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DETERMINATION OF DEXTROSE AND LEVULOSE IN HONEY

COMPARISON OF METHODS[1]

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Although levulose is the most important sugar of honey, both quantitatively and because of its effect upon the physical properties, relatively little attention has been given in recent years to its determination in this product. The method of low- and high-temperature polarization was introduced by Wiley (1) in 1896 and used by Browne in his classical analyses of honey reported in 1908 (2). Although included since the first edition of the "*Methods of Analysis*," A.O.A.C., it is still designated first action in the 1950 edition. The method is strictly applicable only when the rotation of all other substances in a mixture is unaffected by temperature change. It was recognized that this is not strictly true with honey (2).

The fundamental constant upon which this method is based, i.e. the change of rotation per gram of levulose in 100 ml solution in a 2.00 dm tube between 20° and 87°C., was found by Wiley to be 0.0357°V. for the interval 0–88°C. He also found the change to be uniform over this temperature range. Browne and Zerban (3) listed the values for this constant calculated from the data of five investigators previous to Wiley; the average was 0.0362. The value of 0.036 was confirmed by Jackson and Silsbee (4) who reported it to vary somewhat with concentration and details of manipulation. However, later study by Jackson and Mathews (5) using very pure levulose and improved methods, yielded a value of 0.03441 over a concentration range of 3 to 18 per cent and a temperature range of 20° to 70°C. This figure is the average of 30 observations at six concentrations. Lothrop (6) reported an average value of 0.03415 between 20° and 70° for two concentrations.

Tsuzuki and co-workers (7) have determined the specific rotation of

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levulose over the temperature range 10–90°C. and for concentrations of 5 to 40 per cent by weight. Calculations from their values for 10 per cent levulose give, for the change in rotation per gram levulose per degree, the values 0.03449 for 20–90°C. and 0.03454 for 20°–70°.

Jackson and Mathews (5) also reported their determination of the mean expansion coefficient for levulose solutions between 20° and 70° to be 0.00044 ml per ml per degree, rather than the 0.00047 value used by Browne (2) and subsequently by the A.O.A.C. This latter value is the expansion of water over the range.

The polarimetric determination specified by the A.O.A.C. requires the determination of rotation at 20° and 87°C. Jackson and Mathews recommended that readings be made at 20° and 70° when their revised constants are used. It is implied (3) that since the change of rotation of levulose is uniform, readings may be made above 20° for the low temperature value. This would permit somewhat simpler apparatus.

A number of chemical methods for selective determination of dextrose or levulose in honey were studied by Lothrop and Holmes (8). A modification of the Hinton and Macara method for dextrose was developed and applied to honey. In their method dextrose is quantitatively oxidized by hypoiodite under strictly controlled conditions; correction is made for a 1.2 per cent oxidation of levulose, and levulose is calculated by difference from values for total reducing sugar by the Munson and Walker or Lane and Eynon methods. They reported the analysis of 33 floral types of honey by their method and included a comparison of levulose values for ten samples by both oxidation and polarization methods. Consistently higher values (average 1.33 per cent levulose) were obtained by the chemical method. In 1938 Lothrop (6) pointed out that if instead of the A.O.A.C. value, the Jackson-Mathews polarimetric constant of 0.03441 were used to calculate levulose, the average difference in levulose content found by the two methods became 0.19 per cent.

For a selective levulose method, Jackson and Mathews (5) took advantage of the difference in reducing power of levulose against a modified Ost's solution, and against another reagent (Quisumbing and Thomas, or Lane and Eynon). Two simultaneous equations were solved for dextrose and levulose content. This was applied to ten honey samples by Jackson, Mathews, and Chase (9). Expressing a desire for a more accurate, reproducible procedure for the determination of dextrose and levulose in honey, Marshall and Norman (10) preferred a method in which each sugar is determined directly, rather than determination of one sugar directly and the other by difference from a total reducing sugar value. They pointed out that in indirect methods any error in the determination of one sugar affects the other in the opposite direction, resulting in a value for levulose to dextrose ratio that may be far from correct. Determination of levulose by a copper-reduction method after destruction of dextrose was the principle adopted. They selected the Lothrop-Holmes procedure, with slight modification, for the determination of dextrose, and also for its destruction prior to the determination of residual sugars as levulose by the Shaffer-Somogyi method. They studied mutual interference of the two sugars and presented equations for calculation of levulose and dextrose contents. Results of the application of this method to 13 British honeys are listed. Subsequently Ugarte and Karman (11) reported the analyses of 58 Argentine honey samples determined by this procedure.

Hurd *et al.* (12) described the application to honey of their method of determination of sugars by distillation of the propionates. By this procedure the sugars are grouped as mono-, di-, and trisaccharides and no distinction is made between dextrose and levulose. They reported, however, the analysis of six samples of honey by the Jackson and Mathews copper method as well as by the Becker and Englis (13) procedure in which levulose is oxidized by ferricyanide. The former procedure gave levulose to dextrose ratios of less than 1 for three samples, and lower in all cases than by the Becker-Englis method. They suggested that their distillation procedure indicated the presence of a reducing disaccharide as a general component of the samples.

In their description of a colorimetric method for the determination of reducing sugars using triphenyltetrazolium chloride, Mattson and Jensen (14) list analyses of seven honey samples for levulose and dextrose but do not report comparison data by any other method.

Of these publications, that of Lothrop and Holmes gives results of analyzing ten honey samples by more than one procedure, as does that of Hurd *et al.* for six samples. We have applied most of the methods outlined above to the determination of levulose and dextrose in fifteen domestic honey samples representing fourteen floral sources. The A.O.A.C. and Jackson-Mathews polarimetric procedures have been compared; the Lothrop-Holmes method, Jackson-Mathews modified Nyns method, and the Marshall-Norman procedure were also used on the same samples. Several other procedures were given preliminary study. One of them was a combination of the diphenylamine method of Rolf, Surtshin and White (15) with the Shaffer-Somogyi method; another was a combination of the diphenylamine method at 75°C. in which the levulose to dextrose color-production ratio is 64 (15) with a similar procedure at 104°C. in which the color ratio is about 8 (15, 16).

EXPERIMENTAL

PREPARATION OF SAMPLES

Fifteen authentic unheated honey samples in 60-pound containers had been procured by the Kansas Agricultural Experiment Station for a study of the role of honey in baking. These were carefully heated for 30 minutes at 160°F., strained, and sampled. The samples were shipped to this laboratory for analysis. Table 1 shows the floral source, area of production and color classification of the samples.

ANALYTICAL METHODS

1. *High- and low-temperature polarization.*—Each sample was analyzed in duplicate by the A.O.A.C. method outlined for honey (17). A silver-lined 2 dm tube was used, with temperature control to ±0.1°C. In addition to readings at 20° and 87°C., values were obtained at 25° and 70°. The higher temperature readings were taken on different aliquots to avoid the effects of decomposition by heat. Levulose was calculated from these results, using: (a) the Wiley coefficient of 0.0357, the expansion coefficient of 0.00047, and the temperatures 20° and 87°, and (b) the Jackson-Mathews coefficient of 0.0344 and expansion coefficient of 0.00044. Dextrose was calculated by the A.O.A.C. procedure by difference between the levulose values and the total reducing sugar values obtained below.

TABLE 1.—*Honey samples*

NO.	FLORAL SOURCE	LOCALE	MOISTURE[1]	COLOR
			per cent	mm. Pfund
1	Yellow Sweet Clover	Kansas	15.12	25
2	Mesquite	Texas	16.60	32
3	Alfalfa	Arizona	14.92	44
4	Star Thistle	California	15.96	49
5	Tupelo	Florida	18.24	54
6	Eucalyptus	California	17.00	64
7	White Clover	California	15.60	22
8	Orange	California	14.76	21
9	Heartsease	Iowa	16.68	50
10	Horsemint	Texas	15.32	40
11	Spanish-needle	Kansas	17.80	73
12	Buckwheat	New York	15.44	119
13	Fall Flower	New York	17.24	111
14	Alfalfa	California	14.28	53
15	Cotton	Texas	16.04	26

[1] *By refractometer.*

2. *Lothrop-Holmes method.*—Each sample was analyzed in duplicate, using aliquots from the solutions clarified for polarization. Total reducing sugars were determined* by a modified Luft-Schoorl method and levulose was calculated by the procedure of Lothrop and Holmes.

3. *Jackson-Mathews modified Nyns method.*—Each sample was analyzed in duplicate by this procedure. After filtration the precipitated cuprous oxide was determined by the volumetric dichromate method of Jackson and Mathews (5). Direct determination without filtration was not found applicable because of excessive loss of iodine caused by CO_2 evolution upon acidification of the reaction mixture. Total reducing sugars were determined by the Munson and Walker method.

4. *Marshall-Norman method.*—Each sample was analyzed in duplicate by this method.

5. *Other procedures.*—Preliminary studies using known solutions indicated that the diphenylamine procedures previously mentioned were not sufficiently accurate to permit application to the levulose-dextrose system.

* We are indebted to Mrs. P. D. Harper of the Analytical, Physical-Chemical and Physics Division for these analyses.

Table 2.—*Determination of dextrose in honey*

NO.	BY DIFFERENCE			DIRECTLY	
	AOAC	JACKSON-MATHEWS (POLARIMETRIC)	JACKSON-MATHEWS (OXIDATION)	LOTHROP-HOLMES	MARSHALL-NORMAN
1	35.15	33.21	40.87	35.68	36.81
	35.22	32.91	40.95	35.70	36.67
2	36.72	34.66	42.96	37.41	37.10
	37.03	35.95	43.21	37.39	37.49
3	37.84	36.03	41.66	37.87	36.99
	37.98	35.97	41.02	37.97	36.43
4	37.32	35.82	40.05	37.10	35.46
	37.03	35.27	39.97	37.12	35.75
5	29.97	28.11	31.66	29.11	29.37
	30.01	28.27	31.53	28.99	29.30
6	33.92	32.51	38.51	33.87	33.08
	33.73	32.12	38.09	33.64	33.63
7	37.56	35.32	39.74	37.61	37.35
	37.71	35.80	39.14	37.55	37.04
8	34.77	33.23	38.98	35.06	34.15
	34.69	33.38	40.31	35.15	34.48
9	35.74	34.12	41.97	36.24	35.89
	35.79	33.74	41.80	36.22	36.22
10	36.48	36.53	37.37	36.19	36.21
	38.66	36.84	39.58	36.21	36.12
11	33.60	32.01	35.61	32.17	28.30
	33.48	32.19	33.94	32.44	28.18
12	36.46	36.06	33.19	36.34	34.91
	37.32	35.78	32.55	36.70	34.81
13	38.76	37.15	39.73	37.06	36.32
	39.02	37.47	38.47	36.82	36.16
14	35.70	33.65	41.34	37.88	37.35
	35.92	33.84	40.15	37.87	37.99
15	40.04	38.86	39.70	37.89	37.90
	40.02	39.40	39.27	37.64	37.58

TABLE 3.—*Determination of levulose in honey*

NO.	DIRECTLY				BY DIFFERENCE
	AOAC	JACKSON-MATHEWS (POLARIMETRIC)	MARSHALL NORMAN	JACKSON-MATHEWS (OXIDATION)	LOTHROP-HOLMES
1	37.61	39.73	40.83	36.30	39.76
	37.53	40.06	40.89	35.55	39.74
2	38.37	39.62	41.83	36.17	39.61
	38.03	39.21	42.32	36.13	39.63
3	38.32	40.30	40.57	35.78	40.32
	38.17	40.36	40.05	36.57	40.41
4	35.63	37.27	37.85	35.01	37.81
	35.95	37.87	39.32	34.83	37.79
5	41.66	43.74	46.45	39.36	44.46
	41.71	43.56	45.86	39.48	44.59
6	37.90	39.44	41.46	35.98	39.83
	38.11	39.87	41.13	35.93	40.08
7	38.21	40.66	41.31	35.77	40.15
	38.05	40.13	41.78	36.17	40.21
8	38.74	40.43	41.03	38.46	40.36
	38.83	40.36	41.06	38.01	40.27
9	38.16	39.94	42.09	36.38	39.58
	38.11	40.35	42.47	35.97	39.60
10	35.54	37.67	38.94	34.43	36.06
	35.34	37.33	39.06	33.88	36.04
11	40.44	42.18	43.41	37.73	41.65
	40.57	41.98	43.07	37.87	41.36
12	36.44	36.87	36.16	39.32	36.17
	35.50	37.18	36.15	38.92	36.00
13	37.42	39.18	41.09	34.63	39.07
	37.14	38.83	40.72	35.29	39.33
14	38.04	40.28	41.97	36.81	37.63
	37.80	40.08	41.86	37.00	37.64
15	37.77	39.06	41.04	36.65	39.79
	37.79	38.47	41.00	37.25	40.06

RESULTS

The results of the application of these analytical methods to the fifteen honey samples are shown in Tables 2 and 3.

When applied to honey the Wiley method for levulose presupposes not only that the change of rotation for levulose with temperature is constant over the range involved, but also that no other component of honey shows significant change of rotation with temperature. Browne and Zerban (3) state that 1.5 g arabinose, 3.0 g galactose, 7.0 g maltose or 9.0 g lactose show about the same variation of rotation with temperature as 1 g of levulose. Of these sugars, only maltose has been demonstrated in honey (18); Hurd *et al.* (12) state that maltose or another reducing disaccharide is a general component of honey.

The polarimetric data at the four temperatures have been used to calculate the change of rotation (°S) per degree C per g levulose in each

TABLE 4.—*Change in polarization per degree per gram levulose in clarified honey solutions*[a]

TEMPERATURE INTERVAL	$\frac{\Delta P}{\Delta T g}$
20–25°C.	0.0159
25–70	0.0374
70–87	0.0324
20–70	0.0346
20–87	0.0340
20–70[b]	0.0344
20–70[c]	0.0345
20–87[d]	0.0357
20–90[c]	0.0345

[a] Degrees S for solutions of 26 g. honey per 100 ml. in 2 dm. tubes.
[b] Value for levulose by Jackson-Mathews (5).
[c] Values for levulose by Tsusuki et al. (7).
[d] Value for levulose by Wiley (1).

of the clarified solutions from the 30 samples. To provide a value for levulose independent of the saccharimetric data, levulose was calculated for each sample from the Lothrop-Holmes results. Although the results have no absolute significance, the relationships among them are of interest. Table 4 shows the averages of all samples calculated in this manner. It is significant that the change over the 20–25° range is only about 40 per cent of that for the 25–70° interval. The lower value for the 70–87° interval might be ascribed to heat destruction of levulose, but the low value in the 20–25° range must be due to other substances in the honey that do not show a uniform temperature-rotation relationship over the entire range. It can be seen that if the intervals 25–70 or 25–87 are selected for analysis for levulose, considerably higher values will be obtained, since the change over this temperature range is considerably greater than either the Wiley or Jackson-Mathews constants. The agreement of the value for 20–70° in Table 4 with the Jackson-Mathews constant is as expected, since the levulose as determined by the Lothrop-Holmes method and used in this calculation is in general agreement with levulose values calculated from the Jackson-Mathews constant.

Lothrop (6) has compared the average levulose values for ten honeys as determined by the Lothrop-Holmes iodometric method and by low- and high-temperature polarization. In the latter procedure he compared use of the Wiley constant of 0.0357 with the Jackson-Mathews value of 0.0344. Close agreement was demonstrated between levulose values by the iodometric method and those from the optical method using the Jackson-Mathews constant. His results are shown in Table 5. Also shown in this table are similar average values for the 15 honeys analyzed in this study. The agreement between values obtained by these two methods

TABLE 5.—*Average levulose content of honey as found by different methods (per cent)*

	NO. OF HONEYS ANALYZED	LOTHROP-HOLMES IODOMETRIC	POLARIMETRIC		
			AOAC	JACKSON-MATHEWS	DIFFERENCE
		(*A*)		(*B*)	(*B*−*A*)
Lothrop-Holmes (6)	10	40.03	38.70	40.22	0.19
This Research	15	39.58	38.00	39.73	0.15

appears to be similar to that found by Lothrop. It will be shown, however, that the difference is statistically significant.

The polarimetric data and the Lothrop-Holmes analyses were carried out on the same solutions. Results by the other methods were obtained intermittently over the following four months. It is doubted whether any significant change took place in total reducing sugar values by enzymatic action on sucrose, or in the dextrose and levulose content of the samples. Auerbach and Bodlander (19) state that the proportion of levulose in honey apparently increases on storage. Later work by Boer (20) failed to substantiate this for honeys having levulose to dextrose ratios greater than 1.06.

The Jackson-Mathews copper reduction method, as previously noted, seems frequently to give higher dextrose and lower levulose values than other methods (Tables 6 and 7). This method has been included as alternative to the polarimetric method for levulose in honey by the A.O.A.C. since the fifth (1940) edition of *Methods of Analysis*. No data have been found in the literature comparing results of levulose analysis by these two methods.

TABLE 6.—*Analysis of variance for the determination of dextrose in honey by five methods*

NO.	A.O.A.C.	JACKSON-MATHEWS (POLARIMETRIC)	JACKSON-MATHEWS (OXIDATION)	LOTHROP-HOLMES	MARSHALL-NORMAN	TOTALS
1	35.2	33.1	40.9	35.7	36.7	181.6
2	36.9	35.3	43.1	37.4	37.2	189.9
3	37.9	36.0	41.3	37.9	36.7	189.8
4	37.2	35.6	40.0	37.1	35.6	185.5
5	30.0	28.2	31.6	29.0	29.3	148.1
6	33.8	32.3	38.3	33.8	33.3	171.5
7	37.6	35.6	39.4	37.6	37.2	187.4
8	34.7	33.3	39.5	35.1	34.3	176.9
9	35.8	33.9	41.9	36.2	36.0	183.8
10	38.6	36.7	38.5	36.2	36.2	186.2
11	33.5	32.1	34.7	32.3	28.2	160.8
12	36.9	35.9	32.8	36.6	34.9	177.1
13	38.9	37.3	39.1	36.9	36.2	188.4
14	35.8	33.7	40.7	37.8	37.7	185.7
15	40.0	39.1	39.5	37.8	37.7	194.1
Totals	542.8	518.1	581.3	537.4	527.2	2706.8

Calculations for the analysis of variance

	75 ENTRIES	METHODS TOTALS	SAMPLE TOTALS	GRAND TOTAL
Sum of squares	98,383.7	1,467,707.7	490,560.9	7,326,766.2
Divisor	1	15	5	75
Quotient	98,383.7	97,847.2	98,112.2	97,690.2
Subtract	97,690.2	97,690.2	97,690.2	
Sum of squares	693.5	157.0	422.0	

Analysis of variance

VARIANCE ASSOCIATED WITH	BASED ON DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE OR VARIANCE	F	F (5% LEVEL)
Methods	4	157.0	39.25	19.24	2.54
Samples	14	422.0	30.14	14.77	1.88
Experimental error	56	114.4	2.04		
Whole set of 75 measurements	74	693.4			

STATISTICAL ANALYSIS OF DATA

Dextrose.—An analysis of variance was made to determine the extent of the contribution of the five dextrose methods to the total variance, which is the sum of the variances due to both samples and methods. Since the samples were from fourteen different floral sources, differences in their dextrose contents were expected. The averages of duplicate values

Table 7.—*Analysis of variance for the determination of levulose in honey by five methods*

No.	A.O.A.C.	Jackson-Mathews (Polarimetric)	Marshall-Norman	Jackson-Mathews (Oxidation)	Lothrop-Holmes	Totals
1	37.6	39.9	40.9	35.9	39.7	194.0
2	38.2	39.9	42.1	36.1	39.6	195.9
3	38.2	40.3	40.3	36.2	40.4	195.4
4	35.8	37.6	38.6	34.9	37.8	184.7
5	41.7	43.6	46.2	39.4	44.5	215.4
6	38.0	39.7	41.3	36.0	39.9	194.9
7	38.1	40.4	41.5	35.9	40.2	196.1
8	38.8	40.9	41.0	38.2	40.3	199.2
9	38.1	39.1	42.3	36.2	39.6	195.3
10	35.4	37.5	39.0	34.1	36.0	182.0
11	40.5	42.1	43.2	37.8	41.5	205.1
12	36.0	37.0	36.2	39.1	36.1	184.4
13	37.3	39.0	40.9	34.9	39.2	191.3
14	37.9	40.2	41.9	36.9	37.6	194.5
15	37.8	38.8	41.0	36.9	39.9	194.4
Totals	569.4	596.0	616.4	548.5	592.3	2922.6

Calculations for the Analysis of Variance

	75 entries	Methods totals	Sample totals	Grand total
Sum of Squares	114,311.9	1,711,052.9	570,379.8	8,541,590.8
Divisor	1	15	5	75
Quotient	114,311.9	114,070.2	114,075.9	113,887.9
Subtract	113,887.9	113,887.9	113,887.9	
Sum of squares	424.0	182.3	188.0	

Analysis of Variance

Variance associated with	Based on degrees of freedom	Sum of squares	Mean square or variance	F	F (5% level)
Methods	4	182.3	45.58	47.48	2.54
Samples	14	188.0	13.43	13.99	1.88
Experimental error	56	53.7	0.96		
Whole set of 75 measurements	74	424.0			

(Table 2) for the five methods and the fifteen samples were, therefore, treated as a block experiment to provide a means of calculating the samples and methods variances. The average dextrose values, calculations for the analysis of variance, and the analysis of variance are given in Table 6. This table shows that the variance is about equally divided between the methods and the samples. Both the F values of 19.24 for methods and

14.77 for samples are highly significant when compared to their respective critical 5 per cent F values of 2.54 and 1.88. This shows not only that appreciable differences exist among the individual samples, as expected, but also that the dextrose values found for any given honey sample are dependent upon the method used.

To compare the relative precisions of the five methods, Youden's (21) treatment was used, in which the sum of the squares of the differences between duplicate values for each sample for any one method is compared to that of another method chosen as reference. Although the A.O.A.C. method may be considered the standard method, the Lothrop-Holmes values were used as reference, since the following summations were smaller, and hence always in the denominator in the equation:

$$F = \frac{\Sigma d_a^2}{\Sigma d_b^2}, \text{ where } \Sigma d_a^2 > \Sigma d_b^2.$$

Comparison of each of these F values with the critical F value from statistical tables (21) gives the relative precision of the dextrose methods to be as shown in Table 8, where they are ranked in descending order of precision.

Levulose.—As in the case of the dextrose values, Table 7 shows the average percentage levulose values, the calculations for the analysis of variance, and the analysis of variance. In this treatment, the methods account for a greater proportion of the total variance than they do in the

TABLE 8.—*Relative precision of methods for the determination of sugars in honey*

DEXTROSE			
RANK	METHOD	d^2	F VALUE[1]
1	Lothrop-Holmes	0.4137	—
2	A.O.A.C.	1.1766	2.84[2]
3	Marshall-Norman	1.5248	3.68[2]
4	Jackson-Mathews Polarization	3.8282	9.25[2]
5	Jackson-Mathews Oxidation	14.1072	34.10[2]
LEVULOSE			
RANK	METHOD	d^2	F VALUE[1]
1	Lothrop-Holmes	0.3548	—
2	A.O.A.C.	1.4066	3.96[2]
3	Jackson-Mathews Oxidation	3.0819	8.69[2]
4	Marshall-Norman	3.7789	10.65[2]
5	Jackson-Mathews Polarization	4.7541	13.40[2]

[1] $F = \Sigma d_a^2/\Sigma d_b^2$ where d_a is difference between duplicates for method under test and d_b is difference between duplicates for Lothrop-Holmes method.
[2] Greater than the critical 5% level F value of 2.48 (21) and hence significantly less precise.

case of the dextrose values which were obtained by the same methods on the same samples. The F values of 47.48 for methods and 13.99 for samples are highly significant when compared with the respective critical 5% level F values of 2.54 and 1.88. This indicates that the samples do not have the same levulose content and that the levulose values found for a given honey sample are dependent upon the method used.

The duplicate levulose values were used to determine the relative precision of the methods. Table 8 lists the methods in order of precision. As was found for dextrose, the Lothrop-Holmes gave significantly more precise values than any of the four other methods, with the A.O.A.C. method ranking next.

A *t*-test comparison (21) was made between the dextrose and levulose values obtained by the Lothrop-Holmes method and those obtained by the A.O.A.C. method. In this test the differences, d, between the dextrose values obtained by each method for each honey sample are squared, and the standard deviation of the differences is found by the following equation:

$$s_d^2 = \frac{1}{(n-1)} (d_1^2 + d_2^2 + d_3^2 + \cdots + d_n^2 - n\bar{d}^2)$$

where d_1, d_2 etc. = differences between dextrose values obtained for each honey sample, $\bar{d}$ = average difference, and n = the number of samples = 15. Then substitution of the numerical values gives: $s_d^2 = (1/15-1)(22.7801 - 11.6160) = (1/14)(11.1641)$, or $s_d = 0.8929$. This value of the standard deviation of the differences was then substituted in the following equation:

$$t = \frac{\bar{d}\sqrt{n}}{s_d} = \frac{0.88 \times \sqrt{15}}{0.8929}, \text{ or } t = 3.81.$$

Since the value of *t* of 3.81 is higher than the 5% critical *t* value of 2.145 for 14 degrees of freedom, it can be concluded that the dextrose values obtained by the Lothrop-Holmes method were significantly different from those obtained by the A.O.A.C. method.

The same test was applied to the levulose values obtained by the Lothrop-Holmes and by the A.O.A.C. methods. Here the calculated *t* value of 4.53 was again significantly higher than the 5% critical *t* value of 2.145, showing that the levulose values obtained by these two methods were also significantly different. The fact that the Lothrop-Holmes method gave higher levulose values for 14 of the 15 honey samples may also be regarded as significant.

Since it has been stated (6) that the Lothrop-Holmes and the Jackson-Mathews polarimetric method gave comparable results for dextrose and levulose values in honey, the *t*-test, as described above, was applied to the dextrose values obtained by these methods for the fifteen honey samples.

The calculated t value of 3.04 was higher than the 5% critical t value of 2.514; therefore, the dextrose values obtained by two methods were significantly different. Application of the t-test to the levulose values obtained by these two methods yielded a value of 5.74 (critical 5% t value $=2.514$), showing that the levulose values obtained by these two methods were also significantly different. Thus, the apparently close agreement in levulose results by the two methods shown in Table 7 may not signify that the values obtained are the true levulose contents of the samples, even though the principles of the methods differ.

DISCUSSION

The reason for the lower precision obtained with the polarimetric method recommended by Jackson and Mathews must lie in the 70°C. reading since the value for the 20°C. reading was used for both this method and for the A.O.A.C. method.

A possible reason for the lower precision found for the dextrose determination by the Marshall-Norman method when compared with the Lothrop-Holmes procedure may be temperature variation during the oxidation. The sole difference in the dextrose determination by these two methods is that Lothrop and Holmes require 20°C. while Marshall and Norman specify 15 to 18°C. Accordingly the temperatures employed in this study were 20 ± 0.05°C. and 17 ± 1°, respectively.

We cannot determine from these data which of the several methods gives results closest to the actual composition of the sample. Since the determinations are empirical, the superior precision of the Lothrop-Holmes method over the A.O.A.C. method, considered with the smaller equipment requirement and the simplicity of the procedure, indicate the desirability of future comparative work on the determination of dextrose and levulose by these two methods.

SUMMARY

In a comparative study of methods for the determination of sugars in honey, fifteen samples have been analyzed for dextrose and levulose by five methods. Statistical treatment of the results ranks the methods as follows in order of decreasing precision: for dextrose, Lothrop-Holmes, A.O.A.C., Marshall-Norman, Jackson-Mathews (polarimetric) and Jackson-Mathews (oxidation); for levulose, Lothrop-Holmes, A.O.A.C., Jackson-Mathews (oxidation), Marshall-Norman, Jackson-Mathews (polarimetric). Analysis of variance showed that variance due to methods was as great as that due to differences in dextrose and levulose content of the samples from fourteen different floral sources.

Levulose and dextrose values obtained by the Lothrop-Holmes method are significantly different from those obtained by the A.O.A.C. and by the Jackson-Mathews polarimetric methods.

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